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Evaluation of Bone Marrow Aspiration in Pancytopenia

[1]Dr. Mohd Irfan Tuglak, [2]Dr. Sanjay L. Khandekar, [3]Dr. Virendra Khadse

[1]Assistant professor pathology department, SVNGMC, Yavatmal
[2]Associate professor, pathology department SVNGMC Yavatmal
[3]Assistant professor pathology department SVNGMC Yavatmal

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***Corresponding Author**
Dr. Mohd Irfan Tuglak,
Assistant professor pathology department, SVNGMC, Yavatmal

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A B S T R A C T

Background: Pancytopenia is not a disease entity but a triad of findings that may result from various disease processes, primarily or secondarily involving the bone marrow. Bone marrow aspiration plays an important role in identifying the various causes of pancytopenia. Hence the present study was undertaken to evaluate bone marrow findings in pancytopenia cases and causes of pancytopenia on the basis of bone marrow findings. **Method:** Total 50 patients of aged between 18-70 years presented with pancytopenia in the Department of Pathology, at tertiary care teaching hospital in central India during a period from September 2018 to October 2020 were enrolled and evaluated for physical findings, primary hematological investigation and bone marrow aspirations. **Results:** Commonest age group affected was 41-50 years (28%) with male predominance (68%). General weakness (90%) was the chief complaint and pallor (96%) was commonest physical finding. Megaloblastic anemia (48%) was the common cause of pancytopenia, followed by dimorphic anemia (18%). Lowest hemoglobin range was 3gms/dl-3.5gms/dl noted in case of nutritional and megaloblastic anemia. Lowest leukocyte count was 570cells/mm^3 and noted inmegaloblastic anemia. Lowest platelet count was 10,000cells/$mm^3$and noted in nutritional anemia. Hypercellular marrow was found in 41(82%) patients and common cause was megaloblastic anemia. Hypocellular marrow was seen in 4(8%) cases and normocellular marrow was seen in 5(10%) cases. **Conclusion:** Detailed primary hematological investigations along with bone marrow aspiration and biopsy in cytopenic patients is helpful for understanding of the disease process, to diagnose or to rule out the causes of cytopenia and helpful in planning further investigations and management of cytopenic patients.

Keywords: Pancytopenia; Bone marrow aspiration; Pallor; Megaloblastic; Anemia; Dimorphic; Hypercellular; Hypocellular

INTRODUCTION

Pancytopenia is the reduction of all the three formed elements of blood (erythrocytes, leucocytes & platelets), below the normal reference range leading to anemia, leucopenia and thrombocytopenia [1]. Pancytopenia is not a disease itself but a triad of findings that may result from a number of diseases processes–primarily or secondarily involving the bone marrow [2]. However, there are various mechanisms for developing pancytopenia, in which most important is the decrease in hematopoietic cell production (hypocellular marrow). Others include ineffective erythropoiesis, increase peripheral utilization and destruction of cells & bone marrow with malignant infiltration (hypercellular or normocellular marrow) [3, 4]. First step in the diagnosis of a disease is assessing the blood elements [5]. Physical examination findings and peripheral blood picture provide important information in the work up of patients with pancytopenia and help in planning the investigations on bone marrow samples [6].

Bone marrow aspiration is extremely useful in evaluating the cause of pancytopenia by cellularity and cytology in order to prevent grave complications and mortality as the underlying pathology determines the management and prognosis of the patients [7]. India is a large and diverse country and people have different age groups, customs and dietary habits. Therefore the incidence of causes for pancytopenia is difficult and few studies have discussed about it in the Indian scenario [7, 8]. Hence the present study has been undertaken to evaluate the various causes of pancytopenia

and to evaluate clinical signs and symptoms and hematological parameters along with bone marrow aspirate. Thus it would help in planning the diagnostic and therapeutic approach in patients with pancytopenia

MATERIALS AND METHODS

The present study was carried out in the Department of Pathology atTertiary Care Hospital in central India, during a period from September 2018 to October 2020.The cases were selected on the basis of clinical features and laboratory results. Inclusion criteria were presence of all three- hemoglobin < 10gm/dl, total leukocyte count (TLC) < 4000/microL and platelet count <1,50,000/ microL. Patients who have recently received blood transfusions, patients on radiotherapy, cytotoxic drugs, pregnant women and patients with psychiatric illness were excluded from the study. Investigations included hemoglobin, RBC, WBC, platelet count, HIV, HBsAg, bleeding time and clotting time (when required), peripheral smear and bone marrow study were carried out.

Sample collection

2 ml of blood was collected by venepuncture under aseptic precaution in a dry bulb containing ethylene di-amine tetra acetic acid (EDTA) anticoagulant. Samples were processed by an automated autoanalyzer and blood counts with other details were obtained.

Peripheral smear study

The peripheral smear was studied after staining with Leishman's stain. Special stains like periodic acid schiff reagent stain, myeloperoxidase, sudan black and perl's iron stain were used wherever indicated. Smears were examined under microscope for features of- RBC morphology- (type, morphological anemia, immature RBC's, any inclusions), WBC morphology– for differential count, morphology of each cell, immature cells, platelet count and its morphology and any parasites.

Bone marrow aspiration

A written consent from the patient or patent's guardians was obtained prior to the procedure. Patient was given left or right lateral decubitus position. The posterior superior iliac spine was located. Site was prepared, cleaned with antiseptic betadine and spirit. The surrounding area was draped exposing only the aspiration area. Intramuscular atropine was given and xylocaine skin sensitivity test was done prior to the procedure. Skin, subcutaneous tissue and periosteum overlying the selected site was infiltrated with a local anaesthetic such as 2-5 ml 2% lignocaine. Waited until anaesthesia has been achieved and bone marrow aspiration was performed using salah needle. With boring movement bone marrow aspiration needle with a sty-let in place was passed perpendicularly into the cavity of ilium at the centre of the oval posterior superior iliac spine. When the bone had been penetrated sty-let was removed. 2 ml syringe was attached and marrow contents were sucked up for making films. About 0.2 to 0.5 ml of fluid was aspirated. Aspiration needle was then removed and pressure was applied to the site with cotton pads until bleeding stopped. Preparing the films from bone marrow aspirates: The films were made 3-5 cm in length of aspirated marrow using a smooth-edged glass spreader of not more than 2cm width. The marrow fragments were dragged behind the spreader by leaving behind a trail of cells. After procedure was completed pressure bandage was done and patient was instructed to check the site frequently and to report in case of any bleeding. These aspiration smears were dried and stained with Leishman or MGG stain.

Bone marrow biopsy

Biopsy, whenever required was done along with aspiration in the same setting. Bone marrow biopsy needle inserted at bone with stylet then stylet was withdrawn from the needle and the cap was closed, and then was further pushed into the cavity by rotating movements for about 0.5-1cm. The needle was then withdrawn in the reverse rotating direction. A wire probe was inserted at the needle hub on to a sterile gauge. The specimen was fixed in 10% formalin overnight and decalcified in 6% EDTA for 72 hrs. It is then processed similar to histopathological sample and H and E section were studied.

Statistical analysis

The statistical analysis was performed through SPSS for windows (version 16.0). The Chi square test procedure tabulates a variable into categories and computes chi square statistics. This test compares the observed and expected frequencies in each category to test either that all categories contain the same proportion of values or that each category contains a user specified portion of values.

RESULTS

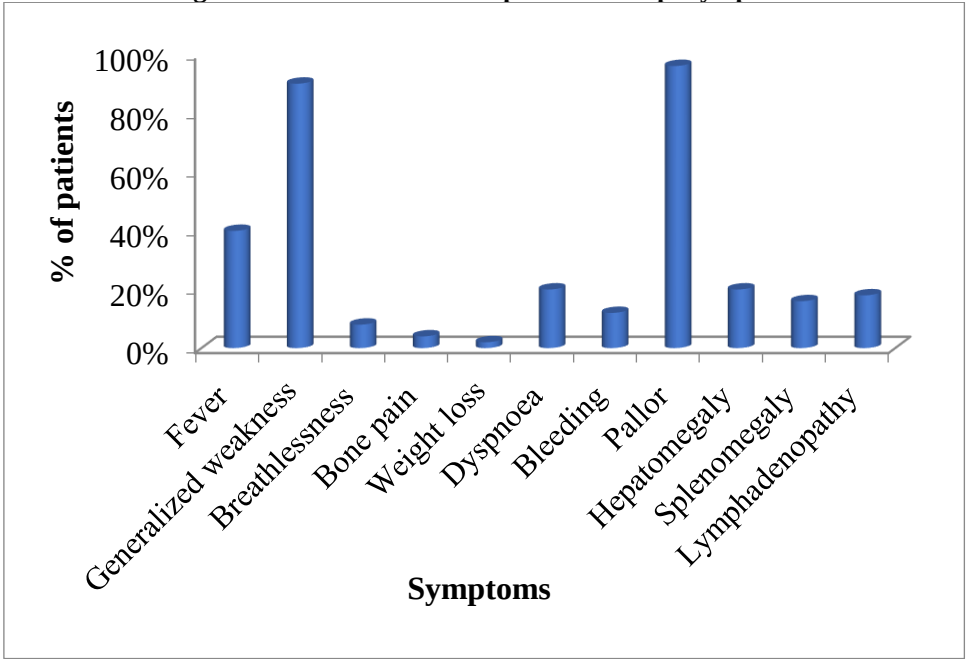
Fifty patients with haematological diagnosis of pancytopenia were enrolled in the study. Most of the patients were in the age group of 41-60 years (54%) and least occurrence was seen in the age group of 18-20(2%)yeas as shown in table 1. There was a male predominance and the male to female ratio was 2.1:1.

Table 1: Age and Sex Groups Distributions in Patients with Pancytopenia

Age group	Females	Males	Total cases	Percentage
18-20	00	01	01	2%
21-30	03	05	08	16%
31-40	04	08	12	24%
41-50	04	10	14	28%
51-60	05	08	13	26%
61-70	00	02	02	4%
Total	16	34	50	100%

Pallor and weakness was the commonest symptom (96% and 90% respectively), followed by breathlessness in 8% of cases and the least was weight loss seen in 2% of the cases as depicted in figure 1.

Figure 1: Clinical features in patients with panytopenia



Majority of the patients had hemoglobin in the range of 5.3- 8% (52%) and leucocyte count in the range of 3100-4000 cells/cumm (46%) whereas platelet count in the range of 51,000-75,000 (42%) as shown in table 2.

Table 2: Hematological Data/ laboratory results

Data		No. of cases	Percentage	P value
Range of hemoglobin	1.8-5%	14	28%	0.045
	5-8%	26	52%	
	8.1-9.6%	10	20%	
Leucocytes count (cells/cumm)	500-1000	03	6%	0.036
	1100-2000	09	18%	
	2100-3000	15	30%	
	3100-4000	23	46%	
Platelet count	4000-25000	01	2%	0.071
	26000-50000	07	14%	
	51000-75000	21	42%	
	76000-1,00,000	15	30%	
	1,01,0000-1,50,000	06	12%	

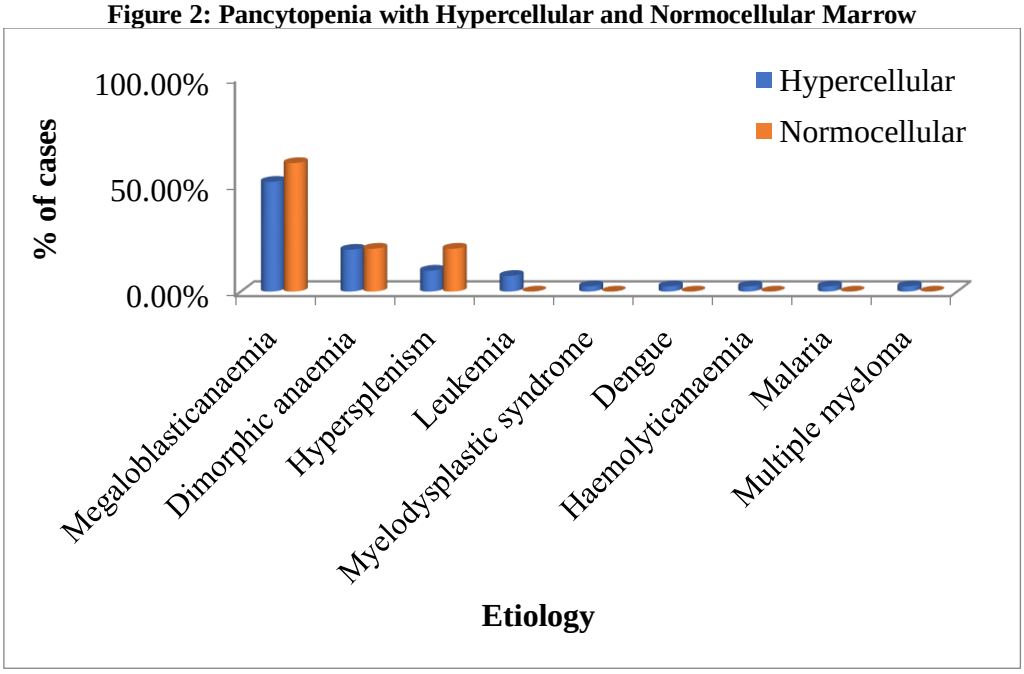
Maximum number of patients had hypercellular marrow (41; 82%) followed by normocellular (5; 10%). However the least number of patients had hypocellularmarrow (4; 8%), ($p<0.000$) and the peak incidence of aplastic anemia was seen in the age group of 41-50 years (2; 50%) with male predominance (male to female ratio was 3:1). The hemoglobin percentage varied from 5 to 9.5%, majority of the cases were in the range of 5-8% (100%) and other details are shown in table 3.

Table 3: Pancytopenia Associated With Hypocellular Marrow

Parameters		No. of cases	Percentage	P value
Age	31-40	01	25%	0.10
	41-50	02	50%	
	51-60	01	25%	
Hemoglobin percentage	1.8-5.0	00	0%	0.886
	5.1-8	04	100%	
	8.1-9.6	00	0%	
Leukocyte count (cells/cumm)	500-1000	00	0%	0.716
	1100-2000	02	50%	
	2100-3000	00	00	
	3100-4000	02	50%	
Platelet count (cells/cumm)	4000-25000	00	00	0.776
	26000-50000	00	00	
	51000-75000	02	50%	
	76000-1,00,000	02	50%	
	1,01,0000-1,50,000	00	00	

The most common etiology noted was megaloblastic anemia (52%), followed by dimorphic anemia (19%), hypersplenism (10%), leukemia and others as depicted in figure 2. Normocellular marrow was seen in megaloblastic anemia (60%), nutritional anemia (20%) and hypersplenism (20%).

Figure 2: Pancytopenia with Hypercellular and Normocellular Marrow



Out of 50, 24(48%) cases of megaloblastic anemia were seen and majority of them were in the age group of 41-50 years (33.33%) with male predominance (male to female ratio- 2.4:1). The majority of patients had hemoglobin values ranging from 5-8% (58.3%), leukocytes in the range of 2100-3000cells/cumm (41.6%), however, most of the patients had platelet count in the range of 51,000- 75,000 (41.66%) as shown in table 4.

Table 4: Pancytopenia with megaloblastic anemia

Parameters	No. of cases	Percentage	P value

Age	21-30	04	16.6	
	31-40	07	29.16	0.18
	41-50	08	33.33	
	51-60	04	16.6	
	61-70	01	4.16	
Hemoglobin percentage	1.8-5.0	06	25	0.046
	5.1-8	14	58.33	
	8.1-9.6	04	16.66	
Leukocyte count (cells/cumm)	500-1000	01	4.16	0.176
	1100-2000	03	12.5	
	2100-3000	10	41.66	
	3100-4000	09	37.5	
	4,100-7000	01	4.1	
Platelet count (cells/cumm)	4000-25000	00	00	0.761
	26000-50000	02	8.33	
	51000-75000	10	41.66	
	76000-1,00,000	08	33.33	
	1,01,0000-1,50,000	04	16.66	

Among 50 patients, 9 (18%) had dimorphic anemia and majority of them were in the age group of 51-60 years (44.4%). The details of hemoglobin values, leucocyte and platelet count in patients with dimorphic anemia are shown in table 5.

Table 5: Pancytopenia with Dimorphic Anemia

Parameters		**No. of cases**	**Percentage**	**P value**
Age	18-20	01	11	0.081
	21-30	01	11	
	31-40	01	11	
	41-50	02	22	
	51-60	04	44.4	
Hemoglobin percentage	1-3	01	11	0.451
	3.1-5	05	55.5	
	5.1-8	02	22	
	8.1-9.6	01	11	
Leukocyte count (cells/cumm)	500-1000	01	11	0.54
	1100-2000	00	00	
	2100-3000	03	33.3	
	3100-4000	05	55.5	
Platelet count (cells/cumm)	4000-25000	01	11	0.876
	26000-50000	03	33.3	
	51000-75000	04	44.4	
	76000-1,00,000	00	00	
	1,01,0000-1,50,000	01	11	

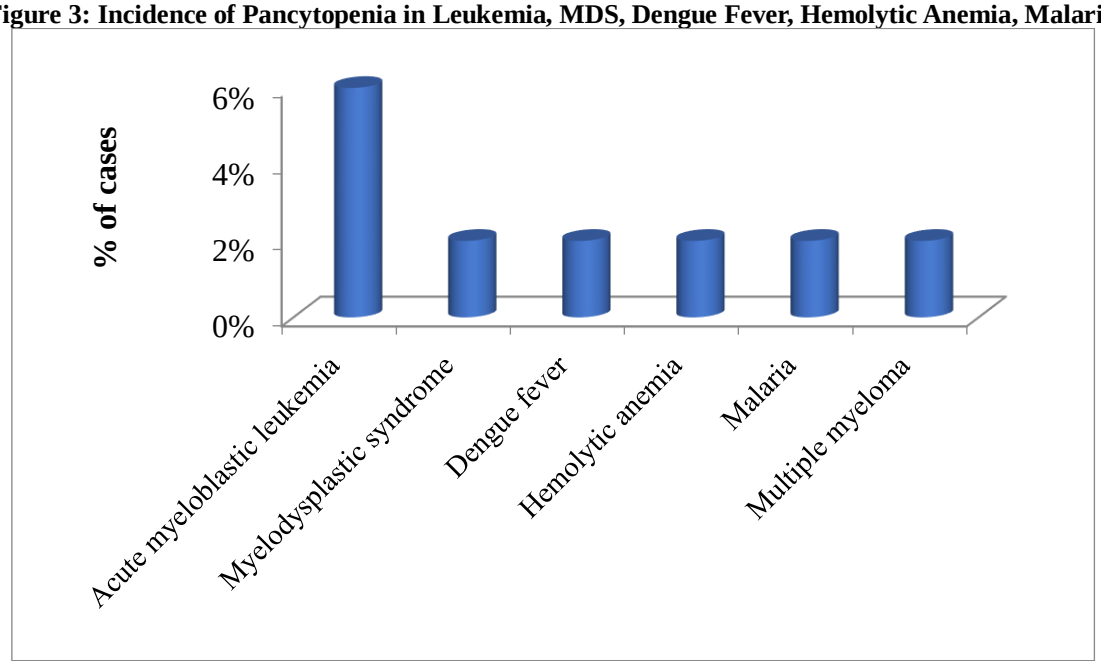
5 (10%) patients of pancytopenia presented with hypersplenism and most of them were in the age group of 51-60 years (60%) with male dominance (male to female ratio 1.5:1). Majority of patients had hemoglobin values ranged from 8.1-9.6% (60%), leucocytes counts in the range of 1100-2000 (60%) and platelet count ranged from 51,000-75,000 and 1, 01,000-1, 50,000 (40%), (Table 6).

Table 6: Pancytopenia with Hypersplenism

Parameters		**No. of cases**	**Percentage**	**P value**
Age	41-50	01	20	0.17
	51-60	03	60	
	61-70	01	20	
Hemoglobin percentage	3.1-5	01	20	0.761
	5.1-8	01	20	
	8.1-9.6	03	60	
Leukocyte	1100-2000	03	60	0.611

count (cells/cumm)	2100-3000	01	20	
	3100-4000	01	20	
Platelet count (cells/cumm)	51000-75000	02	40	0.871
	76000-1,00,000	01	20	
	1,01,0000-1,50,000	02	40	

Figure 3: Incidence of Pancytopenia in Leukemia, MDS, Dengue Fever, Hemolytic Anemia, Malaria



Among 50 patients, 3(6%) patients of pancytopenia showed leukemia as the etiology and majority of them were in the age ranged from 31-40 years (66.6%) with male predominance (male to female ratio 2:1). Maximum patients had hemoglobin values in the ranged of 8.1-9.6% (66.6%) whereas most of the patients had leukocyte count in the range of 3100-4000 (66.6%) and majority of the patients had platelet count ranged from 51,000- 75,000 (66%) as shown in table 7.

Table 7: Pancytopenia with malignant diseases-Acute myeloblastic leukemia

Parameters		**No. of cases**	**Percentage**	**P value**
Age	21-30	01	33	0.018
	31-40	02	66.6	
Hemoglobin percentage	5.1-8	01	33	0.18
	8.1-9.6	02	66.6	
Leukocyte count (cells/cumm)	1100-2000	01	33	0.187
	2100-3000	00	00	
	3100-4000	02	66.6	
Platelet count (cells/cumm)	26,000-50,000	01	33	<1.000
	51,000-75,000	02	66.6	

DISCUSSION

Pancytopenia is frequently encountered hematological problem in various clinical settings all over the world. The recognition of cause of pancytopenia is important in order to come to the accurate diagnosis and proper treatment planning. However pancytopenia is characterized by unexplained anemia, prolonged fever, pallor and generalized weakness as the common presenting symptom as seen in current study. When the patient presents with these findings, pancytopenia should be suspected and further laboratory investigations should be carried out. The physical findings, peripheral blood picture and bone marrow evaluation provides valuable information. The peripheral blood film examination helps in evaluating the most probable cause of anemia while bone marrow evaluation is diagnostic.

In present study, hepatomegaly accounted for 20% of the cases which is comparable with the previous studies [9, 10] while it was higher in other studies [8, 11-13]. Splenomegaly accounted for total 8% of the cases and bleeding was seen in 6% of cases, this is in accordance with the study done by Yadav et al [9] and Khodke et al [13]. Other clinical features were bone pain, weight loss, dyspnoea, breathlessness and lymphadenopathy.

Pancytopenia has many diverse etiological factors, and the occurrence of particular pathology as the entity causing pancytopenia may depend on various factors like genetic constitution, geographical distribution, dietary habits etc. Megaloblastic anemia was the commonest cause which indicates the high prevalence of nutritional anemia in our region and this coincides with the previous studies [8, 10-12, 14]. The other common causes were dimorphic anemia and aplastic anemia. Also anisopoikilocytosis was predominant amongst all the cases. Hypersegmented neutrophils with macro-ovalocytes were commonly found in megaloblastic anemia. Tilak et al [8] and Khunger et al [12] also found similar peripheral blood findings in megaloblastic anemia. Aplastic anemia presented with macrocytosis with normocytic normochromic erythrocytes. Dimorphic hypochromic anemia was reported in 13.5% of the cases. Most of the cases of macrocytic normochromic anemia were confirmed as megaloblastic anemia on bone marrow examination.

Bone marrow evaluation is very helpful in evaluating the cases of pancytopenia. We found majority of the cases to be hypercellular followed by normocellular and hypocellular bone marrow which is similar to Yadav et al study [9]. Most common cause of hypercellular bone marrow was megaloblastic anemia. They had megaloblasts with open chromatin and asynchronicnucleo cytoplasmic ratio in the marrow aspirates. Megaloblasts presented with giant band form appearance, with blasts and mitosis commonly being seen. Other causes of hyper cellular marrow were myelodysplastic syndrome, multiple myeloma and leukemia as confirmed by Khunger et al [12]. Aplastic anemia was the cause of hypocellular marrow, presenting with increased fat cells. Dengue fever on bone marrow aspirations presented with macrophages with engulfed RBCs and lymphocytes. One case presented with increased in plasma cells and plasmablasts in bone marrow aspiration and biopsy, diagnosed as multiple myeloma. One case of myelodysplastic syndrome showed dysplastic changes like, nuclear budding, multinucleation and mitosis. It was similar to cases of myelodysplastic syndrome in Khunger et al [12] and Khodke et al [13]. The hypercellularity of the bone marrow along with abnormal cells confirmed the diagnosis. Hypocellular bone marrow was seen in all the cases of aplastic anemia. Normocellular bone marrow was seen in few cases of megaloblastic anemia, dimorphic anemia and hypersplenism. Thus, bone marrow examination is accurate, reproducible, rapidly available information at an economical cost and with minimal discomfort to the patient. It is sufficient to make a diagnosis in cases of dimorphic anemias and initial diagnosis of leukemia.

We encountered one case (2%) of malaria, leukemia was found in three cases, one case of multiple myeloma and one case of myelodysplastic syndrome, these findings are in accordance with the earlier studies [8, 10-14]. One case of dengue fever was reported in current study, similar to other studies [15, 16]. Also we found one case of hemolytic anemia. So, the uncommon and rare causes such as multiple myeloma, dengue fever, hemolytic anemia and malaria infection should be kept in mind while planning investigation for complete work up of cytopenic patients.

CONCLUSION

The present study concludes that detailed primary hematological investigations along with bone marrow aspiration and biopsy in cytopenic patients is helpful for understanding of the disease process, to diagnose or to rule out the causes of cytopenia and helpful in planning further investigations and management of cytopenic patients**.**

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